

<sup>1</sup>Protein Signaling Domains Laboratory, Biological Sciences, Virginia Tech, Blacksburg, VA, USA. <sup>2</sup>Integrated Cellular Responses Laboratory, Biological Sciences, Virginia Tech, Blacksburg, VA, USA.

To control cellular processes, cells dampen signaling through a broad range of mechanisms including cargo internalization and by slowing down the transport of metabolites through the plasma membrane. The mechanism of cargo removal includes internalization by endocytosis, followed by ubiquitin-mediated delivery into early endosomes. Cargo is further sorted into vesicles of late endosomes/multivesicular bodies followed by, in most cases, degradation in the lysosomal lumen. Adaptor proteins regulate cargo sorting through their ubiquitin-binding domains, ensuring the precise sorting of endosomal ubiquitinated cargo proteins. One of these adaptor proteins is Tollip, which mediates protein sorting by association with Tom1, polyubiquitinated cargo, and clathrin. Tollip is localized on early endosomes where it is required for degradation of ubiquitin-conjugated cargo including the interleukin-1 receptor, a key player in inflammation and innate immunity. Tollip is modular in architecture with an N-terminal Tom1-binding domain (TBD), a central C2 domain, followed by a C-terminal CUE domain. We establish that the Tollip C2 domain preferentially interacts with phosphatidylinositol 3-phosphate (PtdIns(3)P), a phosphoinositide exclusively enriched at the early endosomal membranes. We identified key PtdIns(3)P-interacting residues predicted to be in a flexible region nearby the beta-groove of the protein. We also demonstrate that the CUE domain mediates Tollip's dimerization and binds ubiquitin. More importantly, we establish that ubiquitin negatively regulates Tollip's phosphoinositide binding and this novel function is associated to direct binding to its C2 domain. We also show the structural basis by which the adaptor protein Tom1 binds ubiquitin and Tollip TBD in a mutually exclusive manner. Thus, we propose the association of ubiquitin to Tollip and Tom1 negatively modulates Tollip's membrane targeting. Thereby, our studies provide structural insight of how Tollip is partitioned in different intracellular pools and Tollip's molecular interactions modulate innate immunity and membrane trafficking.

### 337-Pos Board B106

#### Analysis of Integral Membrane Inter and Intra Contacts in Model Multidrug Transporter EmrE using a Bacterial Two-Hybrid Method

Jason C. Burt, Limei Chang, Raymond J. Turner.

University of Calgary, Calgary, AB, Canada.

The bacterial cytoplasmic membrane small multidrug resistance family transporter EmrE consists of four transmembrane helices (TMH) connected by short loops and is considered to multimerize in order to export quaternary cationic compounds from the cytoplasm. Previous consensus on multimerization proposes a facial monomer-monomer interaction located in the C-terminal TMH 4, which is complicated by the mounting evidence of an antiparallel topology dimer and requires additional data on interactions which form dimers or potential higher multimeric states. Bacterial two hybrid method is used as an *in vivo* approach to protein-protein interaction to this end.

In order to characterize regions of interaction between potential parallel or antiparallel dimers, chimeric constructs of full length monomers were made with N- and C-terminal adenylate cyclase domains. Constructs were also made of each TMH topologically constrained with maltose binding protein to the periplasmic side and one of two domains of adenylate cyclase for the bacterial two-hybrid system to the cytoplasmic side. These constructs were then cloned to represent the alternate topology. Complimentary constructs were co-expressed in an *in vivo* system where interaction strength between helices was quantified with the use of the Miller assay. This assay relies on the production of the o-nitrophenol anion by proportional production of  $\beta$ -galactosidase resulting from the transmembrane helix mediated interaction of the adenylate cyclase domains encoded into the chimeric constructs.

Resulting data obtained using full length proteins and individual TMH in different orientations provides information on helix interactions and topology which compliment the *in vitro* structural information.

### 338-Pos Board B107

#### Solute Transport and Selectivity by HpUreI, the Acid-Activated Urea Channel of *H. Pylori* Required for Gastric Survival

Reginald McNulty<sup>1</sup>, Hartmut Luecke<sup>2</sup>, Martin B. Ulmschneider<sup>3</sup>.

<sup>1</sup>The Scripps Research Institute, La Jolla, CA, USA, <sup>2</sup>University of California, Irvine, CA, USA, <sup>3</sup>University of London, London, United Kingdom.

Gastric infection by *Helicobacter pylori* depends on the expression of the urea channel, HpUreI. This inner membrane channel allows *H. pylori* to survive in

low pH environments by funneling gastric urea to the cytoplasmic urease, where it is hydrolyzed into CO<sub>2</sub> and 2NH<sub>3</sub>, which in turn buffer the pathogen's periplasmic space to a pH of 6.1. We have performed microsecond atomic detail molecular dynamics simulations of the recently solved crystal structure of HpUreI (Strugatsky et al. *Nature*, 2012, accepted), to identify the key channel lining residues responsible for solute selectivity. Selectivity through the open channel pore is conveyed by a ring of aromatic residues that form a constriction in the central part of the channel. Urea transport rates through this constriction are restricted by the sidechain rotation periodicities of these residues, which are in the range 100-500 ns, explaining the low solute conduction rate observed experimentally.

Both the channel architecture and conduction mechanism contrast sharply from the urea transporter (UT) channel family. Unlike the UTs, HpUreI has wide water-filled channel pores, that keep urea hydrated during transport, and conduct significant amounts of water.

Strugatsky D, McNulty R, Munson K, Chen C, Soltis SM, Sachs G & Luecke H. Crystal structure of the urea channel from the human gastric carcinogen *Helicobacter pylori*. *Nature* 2012, accepted.

### 339-Pos Board B108

#### Electrophysiology of Ammonium Transport Proteins

Tobias Wacker<sup>1</sup>, Juan J. Garcia-Celma<sup>2</sup>, Philipp Lewer<sup>1</sup>, Susana L.A. Andrade<sup>1</sup>.

<sup>1</sup>Institut für Biochemie Freiburg, Freiburg i.Brsg., Germany, <sup>2</sup>Biochemisches Institut, Universität Zürich, Switzerland.

The transport of ammonium/ammonia across biological membranes is mediated by a family of ubiquitous integral membrane proteins, the Ammonium Transport proteins (Amt) [1]. Current functional interpretations derived from electrophysiology (two-electrode voltage clamp experiments with protein expressed in *Xenopus* oocytes [2,3]), *in vivo* radioactive uptake assays (using methylammonium as alternative substrate [4]) and *in vitro* pH measurements (reported by a pH-sensitive Fluorescence dye inside proteoliposomes [5]), yielded to discrepant results that are still controversially discussed [1,6,7]. To conclude on the substrate identity (NH<sub>4</sub><sup>+</sup> or NH<sub>3</sub>) and the transport mechanism, we are conducting electrophysiology assays on various Amt proteins. Typically, pure protein is reconstituted in liposomes for Planar Lipid Bilayer (PLB) and Solid Supported Membrane (SSM) experiments. So far, assays in PLBs show protein fusion difficulties or weak signals even under macroscopic current recording conditions. On the contrary, SSMs allow testing for higher protein:lipid ratios in a much more stable setup [8]. Under these conditions we have been able to record transient currents upon protein activation with substrate. We are currently further developing this assay to investigate electrogenic ammonium uptake in *Af*-Amt1.

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### 340-Pos Board B109

#### Disulfide Bond Connectivity and Oligomeric State of Anoctamin-1

Gunther Schmalzing<sup>1</sup>, Silvia Detro-Dassen<sup>1</sup>, Ursula Braam<sup>1</sup>,

Manuela Klapperstueck<sup>2</sup>, Fritz Markwardt<sup>2</sup>.

<sup>1</sup>RWTH Aachen University, Aachen, Germany, <sup>2</sup>Martin-Luther-University, Halle, Germany.

TMEM16A(a)/anoctamin-1 (Ano1) has been identified as a Ca-activated chloride channel. Using blue native PAGE, SDS-PAGE and chemical cross-linking, we have shown that the mouse Ano1 channel (mAno1) shares an obligate homodimeric architecture with the hCLC-1 channel (Fallah et al. *Molecular & Cellular Proteomics* 10.1074/mcp.M110.004697, 2011). The homodimeric mAno1 dissociated completely into the protomers in non-reducing SDS buffer, indicating that the protomers are held together by non-covalent interactions and not by intersubunit disulfide bonds.

The mAno1 protomer contains a total of 16 cysteine residues, of which nine are located in the predicted ectodomain and one (C357) in the first transmembrane helix, TM1. To study whether intrasubunit disulfide bonds may shape the assembly-competent conformation of mAno1, we mutated each of the 10 cysteine residue in the ectodomain or the TM individually to alanine. We found that all the C to A single mutants were well expressed and assembled as